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(54) **Composition for and method of preventing or treating breast cancer**

(57) The present invention is a composition for preventing, minimising, or reversing the development or growth of breast cancer. The composition contains a combination of a selective estrogen receptor modulator selected from at least one of raloxifene, droloxifene, toremifene, 4'-iodotamoxifen, and idoxifene and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their respective naturally occurring glucosides and glucoside conjugates. The

present invention also provides a method of preventing, minimising, or reversing the development or growth of breast cancer in which a selective estrogen receptor modulator selected from at least one of raloxifene, droloxifene, toremifene, 4'-iodotamoxifen, and idoxifene is co-administered with at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their naturally occurring glucosides and glucoside conjugates to a woman having or predisposed to having breast cancer.

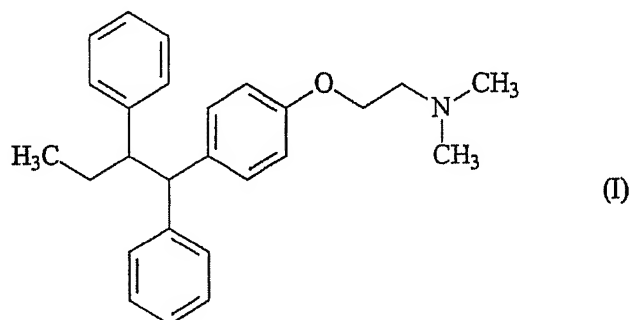
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## Description

[0001] The present invention relates to a composition containing a selective estrogen receptor modulator and at least one isoflavone, and a method of treating breast cancer while inhibiting selective estrogen receptor modulator induced uterotrophic effects.

[0002] Breast cancer is one of the leading causes of cancer mortality among Western women, and is predicted to become a leading cause of cancer death in Oriental women in countries such as Japan in the near future. The American Cancer Society estimates that 1 in 9 women face a lifetime risk of this disease, which will prove fatal for about one-quarter of those afflicted with the disease.

[0003] Tamoxifen, which has the formula (I):



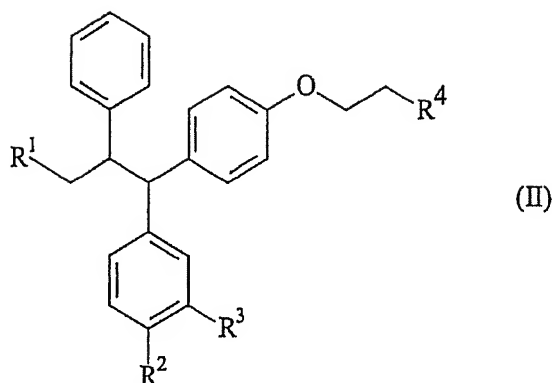
is a synthetic nonsteroidal selective estrogen receptor modulator, and has been used effectively in the treatment of breast cancer for over twenty years. Tamoxifen is one of the most widely prescribed antineoplastic agents in the United States and Great Britain, and is one of the initial hormonal treatments of choice in both premenopausal and postmenopausal women with estrogen receptor positive metastatic disease. Furthermore, adjuvant therapy studies show a substantial reduction of contralateral primary breast carcinoma in tamoxifen treated women, which indicates that tamoxifen may be of use in breast cancer prevention.

[0004] Tamoxifen has tissue-specific estrogenic and anti-estrogenic effects. Estrogen, an ovarian hormone, increases the risk of breast and endometrial cancer by inducing an estrogen receptor mediated increase in the frequency of breast and endometrial cell division. Cell division is essential in the complex process of genesis of human cancer since it per se increases the risk of genetic error - particularly genetic errors such as inactivation of tumour suppresser genes.

[0005] Tamoxifen has anti-estrogenic effects in breast tissue. Tamoxifen's anti-estrogenic effect in breast tissue is a primary mechanism by which tamoxifen inhibits the proliferation of breast cancer cells. Tamoxifen competes with estrogen for binding to cytoplasmic estrogen receptors ("ER"), with subsequent inhibition by the tamoxifen/ER complex of many of the activities of endogenous estrogen within tumour cells. Endogenous estrogen binds with ERs to promote cellular activities such as estrogen/ER-mediated gene transcription, DNA synthesis, cancer cell growth, and increases in autocrine polypeptides such as transforming growth factor-alpha, epidermal growth factor, insulin-like growth factor-II, and other growth factors that may be involved in cell proliferation. Competitive inhibition of estrogen binding to ERs by tamoxifen reduces or prevents such cancer growth inducing cellular activities. As a result of tamoxifen's anti-estrogenic activity in breast tissue, tamoxifen prevents the transition of breast cancer cells from the early G1 phase to the mid-G1 phase of the cell cycle and exhibits a cytostatic effect on breast cancer cells. Tamoxifen has been shown to reduce distant breast cancer metastasis as well as local-regional recurrence of such cancers in both node-negative and node-positive women.

[0006] Tamoxifen, however, has an estrogenic effect on uterine tissues when endogenous estrogen levels are low, which occurs in postmenopausal women and oophorectomized women. Uterine epithelial cell heights are significantly increased by the estrogenic effect of tamoxifen in postmenopausal and oophorectomized women, leading to uterine hypertrophy. Tamoxifen also causes marked uterine eosinophilia. These effects have been associated with endometrial carcinoma, and long term use of tamoxifen is linked to an increased risk of endometrial cancer, up to a fivefold excess of risk relative to women not treated with tamoxifen therapy. Therefore, application of tamoxifen for long term breast cancer prevention and long term treatment of breast cancer has significant associated risks.

[0007] Efforts have been made to develop new selective estrogen receptor modulators ("SERMs") which act in a mechanism similar to that of tamoxifen in breast tissue, while avoiding the risks caused by the estrogenic effects of tamoxifen in uterine tissue. Several of these SERMs are triphenylethylene tamoxifen analogues, which have the formula (II):



in which R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined in Table 1 below:

Table 1

Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
Droloxifene	CH <sub>3</sub>	H	OH	NMe <sub>2</sub>
Toremifene	CH <sub>2</sub> Cl	H	H	NMe <sub>2</sub>
4'-Iodotamoxifen	CH <sub>3</sub>	I	H	NMe <sub>2</sub>
Idoxifene	CH <sub>3</sub>	I	H	pyrrolidino

[0008] As shown above, droloxifene is a tamoxifen analogue in which a 3'-hydroxyphenyl moiety is substituted in place of a phenyl moiety of tamoxifen. Droloxifene has a binding affinity for the estrogen receptor which is ten times that of tamoxifen, has been shown to have anti-estrogenic activity in breast tissue and to be efficacious in treatment of advanced breast cancer, yet has lower estrogenic effects in uterus tissue than tamoxifen. Droloxifene, a New Estrogen Antagonist/Agonist, Prevents Bone Loss in Ovariectomized Rats, Ke et al., *Endocrinology* 136:2435-2441 (1995).

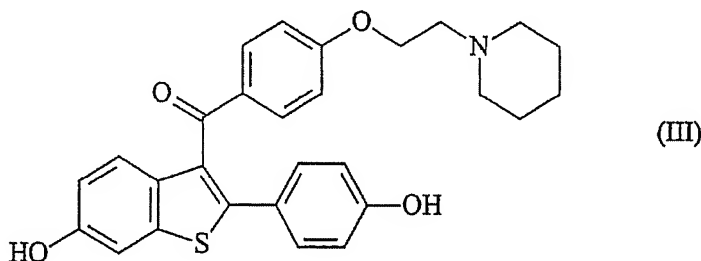
[0009] Toremifene, shown in Table 1, is a tamoxifen analogue having a 4-chloro substituent. Pharmacologically, toremifene has quite similar effects as tamoxifen on breast tissue, acting as potent anti-estrogen. Toremifene also exhibits anti-tumour cytolytic effects at high doses which are independent of its anti-estrogenicity, effects which do not occur with high doses of tamoxifen. Anti-estrogenic Potency of Toremifene and Tamoxifen in Postmenopausal Women, Homesley et al., *Am. J. Clin. Onc.*, 16(2):117-122 (1993).

[0010] 4-Iodotamoxifen, shown in Table 1, is another tamoxifen analogue, having a 4'-iodophenyl substituent in place of a phenyl substituent of tamoxifen. Iodination of tamoxifen at the 4'-phenyl position reduces estrogenic activity, mimicking the high anti-estrogenic activity of the tamoxifen metabolite 4'-hydroxytamoxifen, while giving the compound a longer duration of action *in vivo* by blocking formation of the rapidly metabolised 4'-hydroxytamoxifen metabolite. Pyrrolidino-4-Iodotamoxifen and 4-Iodotamoxifen, New Analogues of the Anti-estrogen Tamoxifen for the Treatment of Breast Cancer, Chander et al., *Cancer Research*, 51:5851-5858 (November 1, 1991); Idoxifene: Report of a Phase I Study in Patients with Metastatic Breast Cancer, Coombes et al., *Cancer Research*, 55:1070-1074 (March 1, 1995). 4-Iodotamoxifen has been shown to have less estrogenic agonist activity in uterine tissue than tamoxifen, and, therefore, is less likely to cause endometrial cancer when administered over a long term.

[0011] Idoxifene, also known as pyrrolidino-4-iodotamoxifen, shown in Table 1, is another tamoxifen analogue, and is modelled on the 4'-iodotamoxifen analogue. Idoxifene has the same general molecular structure as 4'-iodotamoxifen, except that the N,N-dimethylamino moiety of 4'-iodotamoxifen is replaced with a pyrrolidino moiety. Substitution of the pyrrolidino group for the dimethylamino group reduces possible toxic side effects by inhibiting the metabolism of the compound by the liver to a desmethyl metabolite with the concomitant release of potentially toxic formaldehyde. Idoxifene has a 2.5 to 5 fold higher affinity for ERs than tamoxifen, and is 1.5-fold more effective in inhibiting the growth of MCF-7 breast cancer cells. Idoxifene also has less uterotrophic estrogenic effects than tamoxifen and 4'-iodotamoxifen, and produced uterotrophic effects comparable to that of tamoxifen only at a dose which was ten times greater. Pyrrolidino-4-Iodotamoxifen and 4-Iodotamoxifen, New Analogues of the Anti-estrogen Tamoxifen for the Treatment of Breast Cancer, Chander et al., *Cancer Research*, 51:5851-5858 (November 1991); Idoxifene: Report of a Phase I

Study in Patients with Metastatic Breast Cancer, Coombes et al., *Cancer Research*, 55:1070-1074 (March 1, 1995).

[0012] Other SERMs which are not tamoxifen analogues have shown effectiveness in preventing or minimising the development of breast cancer. Raloxifene having the formula (III):



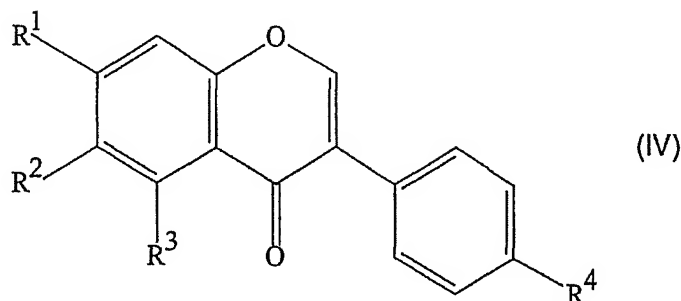
is a benzothiophene derivative, has shown potent anti-estrogenic inhibition of estradiol binding to the ER and significantly inhibits estrogen dependent proliferation of MCF-7 cells derived from human mammary tissue. Raloxifene, unlike tamoxifen and its analogues, exhibits an anti-estrogenic effect in uterine tissue, and provides a nearly complete blockade of uterotrophic responses to estrogen as well as tamoxifen. *Selective Estrogen Receptor Modulators*, Kauffman & Bryant, *DN&P*, 8(9) 531-539 (November 1995).

[0013] It is desirable to utilise these SERMs to develop new compositions which may be used to improve the SERMs' prevention or minimisation of the development of breast cancer while reducing their uterotrophic activity, if any. We have now surprisingly discovered that isoflavones will augment the SERM induced prevention, minimisation, or reversal of the development or growth of breast cancer, as well as prevent or minimise uterotrophic effects associated with some SERMs.

[0014] In one aspect, therefore, the present invention provides a composition for preventing, minimising or reversing the development or growth of breast cancer. The composition comprises a combination of a selective estrogen receptor modulator selected from at least one of raloxifene, droloxifene, toremifene, 4-iodotamoxifen, and idoxifene, and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, or their respective naturally occurring glucosides or glucoside conjugates.

[0015] In another aspect, the present invention is the use of such a combination for the manufacture of a medicament for preventing, minimising or reversing the development or growth of breast cancer in a human. A selective estrogen receptor modulator and an isoflavone are co-administered to a human to prevent or minimise the development or growth of breast cancer. The selective estrogen receptor is selected from at least one of raloxifene, droloxifene, toremifene, 4'-iodotamoxifen, and idoxifene. The isoflavone is selected from at least one of genistein, daidzein, biochanin A, formononetin, or their naturally occurring glucosides or glucoside conjugates.

[0016] Genistein, daidzein, biochanin A, and formononetin may be represented by the following formula (IV):

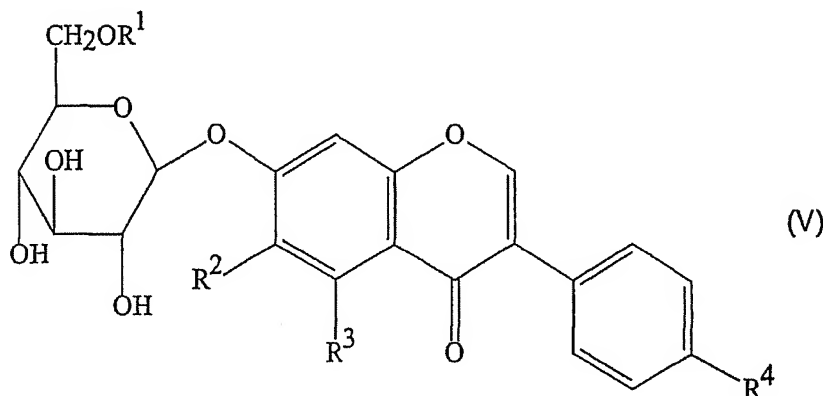


in which R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined in the following Table 2:

Table 2

Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
Genistein	OH	H	OH	OH
Daidzein	OH	H	H	OH
Glycitein	OH	OCH <sub>3</sub>	H	OH
Biochanin A	OH	H	OH	OCH <sub>3</sub>
Formononetin	OH	H	H	OCH <sub>3</sub>

[0017] The naturally occurring glucosides of genistein and daidzein may be represented by the following formula (V):



In which R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined in the following Table 3:

Table 3

Compound	R <sup>1</sup>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
Genistin	H	H	OH	OH
6'-OMal genistin	COCH <sub>2</sub> CO <sub>2</sub> H	H	OH	OH
6'-OAc genistin	COCH <sub>3</sub>	H	OH	OH
Daidzin	H	H	H	OH
6'-OMal daidzin	COCH <sub>2</sub> CO <sub>2</sub> H	H	H	OH
6'-OAc daidzin	COCH <sub>3</sub>	H	H	OH
Glycitin	H	OCH <sub>3</sub>	H	OH
6'-OMal glycitin	COCH <sub>3</sub>	OCH <sub>3</sub>	H	OH

[0018] As used herein, the term "ER" refers to "estrogen receptor". The term "breast cancer" means any cancer having its origin in breast cells, and includes metastatic and local forms of breast cancer (node negative and node positive), as well as ER positive and ER negative forms of breast cancer. The term "uterotrophic effect" means the proliferation of uterine epithelial cells, which frequently is a side effect of administration of selective estrogen receptor modulators to women, and which appears to be directly related to development of endometrial cancer. As used herein "Mal" represents "malonyl" and "Ac" represents "acetyl". The term "minimise", or a derivative thereof, includes a complete or partial inhibition of a specified biological effect (which is apparent from the context in which the term minimise is used). The term "isoflavone" may mean both a single isoflavone or plural isoflavones when the isoflavone is defined as at least one of a selected group of isoflavones. "SERM" means a selective estrogen receptor modulator and its physiologically acceptable salts, other than tamoxifen, which is a compound which produces estrogen antagonist effects in one or more desired target tissues (e.g. breast tissue and uterine tissue), while producing either estrogen agonist effects or minimal agonism in other non-target tissues.

[0019] The present invention resides in the discovery that the combination of selected SERMs with certain isoflavones can be used to treat or prevent breast cancer in a woman having or predisposed to breast cancer, and the isoflavones

will augment the SERM induced prevention, minimisation, or reversal of the development or growth of breast cancer, as well as prevent or minimise uterotrophic effects associated with some SERMs. The SERMs which are useful in the compositions and methods of the present invention are droloxifene, toremifene, 4'-iodotamoxifen, idoxifene, and raloxifene. The isoflavones which are useful in the compositions and methods of the present invention are genistein, daidzein, glycitein, biochanin A, formononetin, their naturally occurring glycosides and their naturally occurring glycoside conjugates, of formulae (IV) and (V).

[0020] The selective estrogen receptor modulator compounds used in the compositions and methods of the present invention can be chemically synthesised according to known methods, and include the salt forms of each of the compounds. Raloxifene, 6-hydroxy-2-(4-hydroxyphenyl)-3-[4-(2-piperidinoethoxy)benzoyl]benzo[b]thiophene (III), and its physiologically acceptable salts may be produced according to the methods described in U.S. Patent Nos. 4,418,068 and 4,133,814. Droloxifene, *E*-1-[4'-(2-dimethylaminoethoxy)phenyl]-1-(3'-hydroxyphenyl)-2-phenyl-1-butene (II, Table 1), and its physiologically acceptable salts may be produced according to the methods described in U.S. Patent No. 5,047,431. Toremifene, 4-chloro-1,2-diphenyl-1-[4-[2-(*N,N*-dimethylamino)ethoxy]-phenyl]-1-butene (II and Table 1), and its physiologically acceptable salts may be produced by the methods described in U.S. Patent No. 4,696,949. 4'-Iodotamoxifen, *E*-1-[4-[2-(dimethylamino)ethoxy]-phenyl]-1-(4-iodophenyl)-2-phenyl-1-butene (II and Table 1), and its physiologically acceptable salts may be produced according to combined methods described in Stereoselective Olefin Formation from the Dehydration of 1-(*p*-Alkoxyphenyl)-1,2 diphenylbutan-1-ols: Application to the Synthesis of Tamoxifen, McCague, *J. Chem. Soc. Perkin Trans.*, 1:1011-1015 (1987); and Derivatives of Tamoxifen. Dependence of Antiestrogenicity on the 4-Substituent, McCague et al., *J. Med. Chem.*, 32(12):2527-2533 (1989). Idoxifene, *E*-1-(4-iodophenyl)-1-[4-(2-pyrrolidinoethoxy)-phenyl]-2-phenyl-1-butene (II and Table 1), may be produced according to combined methods described in the references above that provide methods for producing 4'-iodotamoxifen.

[0021] The isoflavone compounds used in the compositions and methods of the present invention are naturally occurring substances which may be found in plants such as legumes, clover, and the root of the kudzu vine (*pueraria* root). Common legume sources of these isoflavone compounds include soy beans, chick peas, and various other types of beans and peas. Clover sources of these isoflavone compounds include red clover and subterranean clover. Soy beans are a particularly preferred source of the isoflavone compounds (except biochanin A which is not present in soy).

[0022] The isoflavone compounds may be isolated from the plant sources in which they naturally occur, or may be synthetically prepared by processes known in the art. For example, daidzein may be isolated from red clover as disclosed by Wong (*J. Sci. Food Agr.*, Vol. 13, p. 304 (1962)) or may be isolated from the mould *Micromonospora halophytica* as provided by Ganguly and Sarre (*Chem. & Ind. (London)*, p. 201 (1970)). Daidzein may be synthetically prepared by the methods provided by Baker et al (*J. Chem. Soc.*, p. 274 (1933)), Wesley et al. (*Ber.* Vol. 66, p. 685 (1933)), Mahal et al. (*J. Chem. Soc.*, p. 1769 (1934)), Baker et al. (*J. Chem. Soc.*, p. 1852 (1953)), or Farkas (*Ber.* Vol. 90, p. 2940 (1957)). The isoflavone glucoside daidzin may be synthetically prepared by the method of Farkas et al. (*Ber.*, Vol. 92, p. 819 (1959)). The daidzein isoflavone glucoside conjugates 6'-O-Mal daidzin and 6'-O-Ac daidzin can be prepared by a conventional saponification of daidzin with a malonyl or an acetyl anhydride, respectively.

[0023] Genistein may be synthetically prepared by the methods provided by Baker et al (*J. Chem. Soc.*, p. 3115 (1928)); Narasimhachari et al. (*J. Sci. Ind. Res.*, Vol. 12, p. 287 (1953)); Yoder et al., (*Proc. Iowa Acad. Sci.*, Vol. 61, p. 271 (1954); and Zemplen et al. (*Acta. Chim. Acad. Sci. Hung.*, Vol. 19, p. 277 (1959)). The isoflavone glucoside genistin may be synthetically prepared by the method of Zemplen et al. (*Ber.*, Vol 76B, p. 1110 (1943)). The isoflavone glucoside conjugates of genistein, 6'-O-Mal genistin and 6'-O-Ac genistin, can be prepared by a conventional saponification of genistin with a malonyl or an acetyl anhydride, respectively.

[0024] Biochanin A can be synthetically prepared by the method provided by Baker et al. (*Nature* 169:706 (1952)). Biochanin A can also be separated from red clover by the method provided by Pope et al. (*Chem. & Ind. (London)* p. 1092 (1953)). Formononetin can be synthetically prepared by the methods disclosed by Wessely et al. (*Ber.* 66:685 (1933)) and Kagel et al. (*Tetrahedron Letters*, p. 593 (1962)). Formononetin can be isolated from soybean meal by the method of Walz (*Ann.* 489:118 (1931)) or can be isolated from clover species by the method of Bradbury et al. (*J. Chem. Soc. p.* 3447 (1951)).

[0025] It is preferred to extract the isoflavones useful in the compositions and methods of the present invention from the plant materials in which they naturally occur. A preferred method of isolating the isoflavone compounds is to extract the plant materials with an alcohol, preferably methanol or ethanol, or an aqueous solution, preferably an aqueous alkaline solution, to remove the isoflavones from the plant material. It is preferred to comminute the plant material before extracting the isoflavone compounds to maximise recovery of the isoflavone compounds from the plant material. The isoflavone compounds can be isolated from the extract by conventional separation procedures such as reverse phase high performance liquid chromatography ("HPLC").

[0026] In a preferred embodiment, the isoflavone compounds genistein, genistin, 6'-O-Mal genistin, 6'-O-Ac genistin, daidzein, daidzin, 6'-O-Mal daidzin, 6'-O-Ac daidzin, glycitein, glycitin, and 6'-O-Mal glycitin are isolated from a soy material, preferably a commercially available soy material. Soy materials from which the isoflavone compounds can be isolated include: soy beans, dehulled soy beans, soy meal, soy flour, soy grits, soy flakes (full fat and defatted), soy

cotyledons, soy molasses, soy protein concentrate, soy whey, soy whey protein, and soy protein isolate. In one embodiment, the isoflavones are extracted from soy beans, dehulled soy beans, soy meal, soy flour, soy grits, soy flakes, soy protein concentrate, soy whey protein, or soy protein isolate, preferably soy meal, soy flour, soy grits, or soy flakes, with a low molecular weight organic extractant, preferably an alcohol, ethyl acetate, acetone, or ether, and most preferably aqueous ethyl alcohol or methyl alcohol. Most preferably the extractant has a pH at about the isoelectric point of soy protein (about pH 4 to pH 5) to minimise the amount of soy protein extracted by the extractant.

[0027] The extractant containing the isoflavones is separated from the insoluble soy materials to form an isoflavone enriched extract. If desired, an isoflavone enriched material may be recovered by concentrating the extract to remove the solvent, thereby producing a solid isoflavone enriched material.

[0028] In a more preferred embodiment the isoflavone compounds are further purified from other soy materials soluble in the extract by contacting the extract with a material which adsorbs the isoflavones in the extract, and eluting the adsorbed isoflavones out of the adsorbent material with a solvent which causes the isoflavones to be differentially eluted from the adsorbent material.

[0029] In a preferred embodiment, the isoflavones are separated from impurities in the extract by a conventional reverse phase HPLC separation. After extraction of the isoflavones from the soy material and separation of the extract from the insoluble soy materials, the extract is filtered to remove insoluble materials that could plug an HPLC column. An HPLC column is prepared by packing a conventional commercially available HPLC column with a particulate adsorbent material which will releasably bind the isoflavones and impurities in the extract in a compound specific manner. The adsorbent material may be any reverse phase HPLC packing material, however, a preferred packing material may be chosen by the criteria of load capacity, separation effectiveness, and cost. One such preferred packing material is Kromasil C18 16µm 100Å beads available from Eka Nobel, Nobel Industries, Sweden.

[0030] The filtered extract is passed through the packed HPLC column until all the binding sites of the column are fully saturated with isoflavones, which is detected by the appearance of isoflavones in the effluent from the column. The HPLC column may then be eluted with a solvent to effect the separation. In a preferred embodiment, the eluent is a polar solvent such as ethanol, methanol, ethyl acetate, or acetonitrile, and preferably is an aqueous alcohol having an alcohol content of between about 30% and about 90 %, most preferably about 50%, and most preferably the alcohol is ethanol.

[0031] The isoflavone compounds and impurities are separately collected from the column effluent. The isoflavone fractions of the eluent may be identified from other eluent fractions in accordance with conventional HPLC and analytical chemistry techniques. In a preferred embodiment the eluent fractions containing the aglucone isoflavones are collected separately since the aglucone isoflavones are believed to be particularly active tyrosine kinase inhibitors and anti-angiogenesis agents which inhibit the development or progression of breast cancer. Of the aglucone isoflavone materials, the fraction of effluent containing daidzein elutes from the column first, followed by a glycitein fraction, followed by the more polar genistein.

[0032] The isoflavone fractions of the eluent may be collected from the column, and the volatile content of the solvent (e.g. alcohol) can be removed by evaporation. The isoflavone compounds can be recovered directly if all of the solvent is removed by evaporation, or may be recovered by chilling the remaining solvent (e.g. water) to crystallise the isoflavones and centrifuging or filtering the remaining solvent away from the crystallised isoflavones.

[0033] In a particularly preferred embodiment the soy isoflavone glucosides and isoflavone glucoside conjugates -- 6'-O-Mal genistin, 6'-O-Ac genistin, 6'-O-Mal daidzin, 6'-O-Ac daidzin, 6'-O-Mal glycitin, genistin, daidzin, and glycitin -- are converted to their respective aglucone isoflavone forms -- genistein, daidzein, and glycitein. The conversion of the isoflavone glucoside conjugates and the isoflavone glucosides to the aglucone isoflavones can be effected in the substrate from which the isoflavones are to be extracted prior to the extraction, or may be effected in the isoflavone enriched extract after separation of the extract from the insoluble materials. The aglucone isoflavone compounds are especially desirable in the compositions and methods of the present invention since, as noted above, they are believed to be particularly active in inhibiting angiogenesis and inhibiting tyrosine kinase activity.

[0034] The isoflavone glucoside conjugates 6"-O-Mal genistin, 6"-O-Ac genistin, 6"-O-Mal daidzin, 6"-O-Ac daidzin, and 6"-O-Mal glycitin can be converted to their respective glucosides genistin, daidzin, and glycitin by forming an aqueous alkaline solution of the substrate containing the isoflavones having a pH of about 6 to about 13, preferably about pH 9 to about pH 11, and treating the aqueous alkaline solution at a temperature of about 2°C to about 121°C, preferably about 25°C to about 75°C, for a period of time sufficient to effect the conversion, preferably about 30 minutes to about 5 hours, more preferably about 30 minutes to about 1.5 hours. The isoflavone glucosides genistin, daidzin, and glycitin can be converted to their respective aglucone forms genistein, daidzein, and glycitein by contacting the isoflavone glucosides with an enzyme capable of cleaving a 1,4-β-glucoside bond -- preferably a commercially available beta-glucosidase enzyme, an alpha- or beta-galactosidase enzyme, a pectinase enzyme, a lactase enzyme, or a glucosylase enzyme -- at a pH at which the enzyme is active, typically from about pH 3 to about pH 9, and at a temperature of about 25°C to about 75°C, more preferably about 45°C to about 65°C, for a period of time sufficient to effect the conversion, typically about 1 hour to about 24 hours, preferably about 1 hour to about 3 hours.

[0035] The aglucone isoflavones can be separated from the substrate using conventional separation procedures. For example, the aglucone isoflavones may be extracted from the substrate with a low molecular weight alcohol. The aglucone isoflavones may be separated from the extract by conventional recrystallisation processes, or by HPLC. In a particularly preferred embodiment, an isoflavone composition isolated from a soy substrate for formulation into a composition for use in the method of the present invention includes at least 40% genistein, at least 15% daidzein, and at least 1% glycitein. In another particularly preferred embodiment of the invention, an isoflavone composition isolated from a soy substrate for formulation into a composition for use in the method of the present invention contains at least 85% genistein, at least 5% daidzein, and at least 0.5% glycitein. In yet another preferred embodiment, each isoflavone is recovered separately in pure form.

[0036] Several of the isoflavone compounds are commercially available, and may be purchased for formulation into compositions provided in the present invention, or used in the methods of the present invention. For example, genistein, daidzein, and glycitein are commercially available and may be purchased, for example, from Indofine Chemical Company Inc., P.O. Box 473, Somerville, New Jersey 08876, and biochanin A is available from Aldrich Chemical Company, Inc., 940 West Saint Paul Avenue, Milwaukee, Wisconsin 53233.

[0037] In one aspect the present invention is a method for preventing or minimising the development or growth of breast cancer in a human by co-administering at least one SERM selected from raloxifene, droloxifene, toremifene, 4-iodotamoxifen, and idoxifene, and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, their respective glucosides, and their respective glucoside conjugates. The SERM and isoflavone may be co-administered prophylactically to prevent the development of breast cancer in women susceptible of developing breast cancer, or the SERM and isoflavone may be co-administered to treat breast cancer by preventing, minimising, or reversing the growth and development of the cancer. The SERM may be obtained for use in accordance with the method the present invention as described above, or, preferably, may be provided in a composition of the present invention, as described below. The isoflavone may be obtained for use in accordance with the method of the present invention as described above, or, preferably, may be provided in a composition of the present invention, as described below.

[0038] The SERM and the isoflavone may be co-administered either concurrently or sequentially within a specified period of time, preferably daily, on a periodic basis. Most preferably the SERM and the isoflavone are co-administered concurrently in a composition of the present invention, as described below, on a periodic basis, preferably daily. Alternatively, the SERM and the isoflavone are administered sequentially as separate components. "Sequentially" as used herein is intended to mean administration of desired amounts of the SERM and isoflavone individually within a specified periodic period of time, for example daily, and is not intended to be limited to immediate consecutive administration of the SERM and isoflavone.

[0039] The SERM is administered in an amount sufficient to prevent or treat the development or growth of breast cancer in combination with the isoflavone. The amount of SERM sufficient to prevent or treat the development or growth of breast cancer in combination with the isoflavone is dependent on the particular SERM utilised, the amount and activity of the isoflavone utilised, the size of the patient to which the SERM is administered, whether the SERM is administered prophylactically or to treat breast cancer, and if used in treatment, the extent of the cancer. The amount of SERM sufficient to prevent the development of breast cancer in a woman predisposed to breast cancer is preferably at least 0.5 mg per day, more preferably from about 0.5 mg to about 100 mg per day, and most preferably from about 5 mg to about 50 mg per day. The amount of SERM sufficient to treat the development or growth of breast cancer to prevent, minimise, or reverse the development or growth of the cancer is preferably at least 0.5 mg per day, more preferably from 0.5 mg to about 500 mg per day, and most preferably from about 40 mg to about 400 mg per day. The SERM may be administered in several doses per day to achieve the daily amount of the SERM sufficient to prevent or treat breast cancer, however, it is preferred that the daily required amount of SERM be administered in one or two doses.

[0040] The isoflavone is co-administered to the human in an amount sufficient to prevent or treat the development or growth of breast cancer in combination with the SERM. The amount of isoflavone sufficient to prevent or treat the development or growth of breast cancer in combination with the SERM is dependent on the particular isoflavone utilised, the amount and activity of the co-administered SERM, the size of the patient, whether the isoflavone is administered prophylactically or to treat breast cancer, and if used in treatment, the extent of the cancer. The amount of isoflavone sufficient to prevent the development of breast cancer in a woman predisposed to breast cancer in the present method is preferably at least 1 mg per day, more preferably from about 10 mg to about 200 mg per day. The amount of isoflavone sufficient to treat the development or growth of breast cancer to prevent, minimise, or reverse the development or growth of the cancer is preferably at least 1 mg per day, more preferably from about 1 mg to about 1000 mg per day, and most preferably from about 50 mg to about 500 mg per day.

[0041] The isoflavones utilised in the method of the present invention prevent, minimise, or reverse the growth of breast cancer by several mechanisms, which in combination with the anti-estrogenic activity of the SERM in breast tissue, increase the relative anti-breast cancer activity of each compound. First, the isoflavones are anti-estrogenic in



breast tissue, and serve to competitively inhibit estrogen induced cancerous breast cell division by binding to the ER of the cell, where the isoflavone/ER complex inhibits cancer cell growth in much the same manner as tamoxifen and the SERMs (e.g. daidzein halts cell growth in the G1 phase of the cell cycle, and genistein halts cell growth in the G2 phase of the cell cycle). Second, some of the isoflavones, particularly genistein and biochanin A, and to a lesser extent daidzein and formononetin, are tyrosine kinase inhibitors which inhibit enzymatic tyrosine kinase activity. Tyrosine kinase activity is necessary for cancerous cells to produce proteins required for cellular differentiation and growth. Third, the isoflavones inhibit angiogenesis, thereby preventing a cancerous cell mass from developing the network of blood vessels necessary to support the cell mass, limiting the sustainable growth of the cell mass. Fourth, the isoflavones decrease endogenous estrogen levels by interfering with pituitary and hypothalamus gland feedback mechanisms which regulate the release of gonadotropins such as estradiol. The effect of the combined mechanisms of action is to further prevent or minimise the development or growth of breast cancer when co-administered with a SERM effective to prevent or minimise the growth of breast cancer.

[0042] In a particularly preferred embodiment of the method of the present invention, the isoflavone is co-administered with the SERM in an amount sufficient to prevent or minimise SERM induced uterotrophic effects. Although the SERMs utilised in the present invention are less uterotrophic than tamoxifen, each of the SERMs except raloxifene induces uterotrophic effects at relatively high doses. The isoflavones utilised in the present method have an anti-estrogenic effect in uterine tissues when concentrations of estrogen or an estrogen agonist SERM are relatively high. One mechanism by which the isoflavones likely cause an anti-estrogenic effect in uterine tissue in the presence of uterine estrogen agonist SERMs is by binding to uterine cell ERs and competitively inhibiting the estrogen agonist SERMs from binding to the ERs. Unlike uterine tissue estrogen agonist SERMs, the isoflavones do not cause an estrogenic response upon binding to uterine cell ERs, therefore, the isoflavones prevent, inhibit, or minimise the uterotrophic effects caused by uterine endothelial cell ER/SERM complexes. Preferably the isoflavone is co-administered with the SERM to prevent or minimise uterotrophic effects in a weight/weight ratio of isoflavone:SERM of about 0.25:1 to about 100:1, and more preferably from about 0.5:1 to about 20:1.

[0043] In a particularly preferred embodiment of the method, co-administration of the isoflavone with a uterine tissue estrogen agonist SERM in an amount sufficient to prevent or minimise uterotrophic effects is also effective to prevent or minimise the development of endometrial cancer when the SERM is used to prevent or treat breast cancer. As noted above, tamoxifen and uterine tissue estrogen agonist SERMs cause an increased risk of the development of endometrial cancer as a result of estrogen-like activity in uterine tissue and its uterotrophic effects. Co-administration of the isoflavone together with an uterine tissue estrogen agonist SERM prevents or minimises the development of endometrial cancer by preventing or minimising SERM induced uterotrophic effects.

[0044] In another aspect, the present invention is a composition useful for preventing or minimising the development or growth of breast cancer. The composition includes combination of a selective estrogen receptor modulator selected from at least one of raloxifene, droloxifene, toremifene, 4-idotamoxifen, and idoxifene, and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, their respective naturally occurring glucosides and glucoside conjugates. These SERM and isoflavone materials necessary to form compositions in accordance with the present invention may be obtained as described above. The composition contains from about 1% to about 99% SERM, by weight of biologically active ingredients, and from about 1% to about 99% isoflavone, by weight of biologically active ingredients.

[0045] The SERM is present in the composition in an amount sufficient to prevent, minimise, or reverse the development or growth of breast cancer in a woman when co-administered with the isoflavone. Preferably at least 0.5 mg of the SERM is present in the composition, more preferably from about 0.5 mg to about 500 mg, and most preferably from about 5 mg to about 100 mg. Most preferably, the SERM is present in the composition in an amount sufficient to prevent, minimise, or reverse the development or growth of breast cancer by itself.

[0046] Preferably at least 1 mg of the isoflavone is present in the composition, more preferably from about 1 mg to about 1000 mg, and most preferably from about 10 mg to about 200 mg. In a preferred embodiment the isoflavone is present in the composition in an amount sufficient to augment the composition's SERM induced prevention or minimisation of development or growth of breast cancer when the composition is administered to a woman. In a more preferred embodiment, the isoflavone is present in the composition in an amount sufficient to prevent, minimise, or reverse the development or growth of breast cancer by itself.

[0047] In another preferred embodiment, the isoflavone is present in the composition in an amount sufficient to prevent or minimise the composition's SERM induced uterotrophic effects when the composition is administered to a woman. The isoflavone should be present in a ratio of isoflavone:SERM of from about 0.25:1 to about 100:1 by weight, and more preferably from about 0.5:1 to about 50:1 by weight, to be present in the composition in an amount sufficient to prevent or minimise the composition's SERM induced uterotrophic effects. In a most preferred embodiment, the isoflavone is present in the composition in an amount sufficient to augment the composition's SERM induced prevention or minimisation of the development or growth of breast cancer and to prevent or minimise the composition's SERM induced uterotrophic effects when the composition is administered to a woman.

[0048] A composition in accordance with the present invention containing a SERM and an isoflavone can be prepared by conventional procedures for blending and mixing compounds. Preferably, the composition also includes an excipient, most preferably a pharmaceutical excipient. Compositions containing an excipient and incorporating the SERM and isoflavone can be prepared by procedures known in the art. For example, the SERM and the isoflavone can be formulated into tablets, capsules, powders, suspensions, solutions for parenteral administration including intravenous, intramuscular, and subcutaneous administration, and into solutions for application onto patches for transdermal application with common and conventional carriers, binders, diluents, and excipients.

[0049] Inert pharmaceutically acceptable carriers useful to form pharmaceutical compositions in accordance with the present invention include starch, mannitol, calcium sulphate, dicalcium phosphate, magnesium stearate, sillic derivatives, and/or sugars such as sucrose, lactose, and glucose. Binding agents include carboxymethyl cellulose and other cellulose derivatives, gelatin, natural and synthetic gums including alginates such as sodium alginate, polyethylene glycol, waxes and the like. Diluents useful in the invention include a suitable oil, saline, sugar solutions such as aqueous dextrose or aqueous glucose, and glycols such as polyethylene or polypropylene glycol. Other excipients include lubricants such as sodium oleate, sodium acetate, sodium stearate, sodium chloride, sodium benzoate, talc, and magnesium stearate, and the like; disintegrating agents including agar, calcium carbonate, sodium bicarbonate, starch, xanthan gum, and the like; and adsorptive carriers such as bentonite and kaolin. Colouring and flavouring agents may also be added to the pharmaceutical compositions.

[0050] The following non-limiting formulations illustrate pharmaceutical compositions of the present invention.

## **FORMULATIONS**

[0051] The following Formulations 1-4 illustrate pharmaceutical formulations including a SERM and an isoflavone.

### **Formulation 1**

#### **Gelatin capsules**

[0052] Hard gelatin capsules are prepared using the following ingredients: SERM 0.5-100 mg/capsule; Isoflavone 0.1-1000 mg/capsule; Starch, NF 0 - 600 mg/capsule; Starch flowable powder 0 - 600 mg/ capsule; Silicone fluid 350 centistokes 0 -20 mg/capsule. The ingredients are mixed, passed through a sieve, and filled into capsules.

### **Formulation 2**

#### **Tablets**

[0053] Tablets are prepared using the following ingredients: SERM 0.5-100 mg/tablet; Isoflavone 0.1-1000 mg/ tablet; Microcrystalline cellulose 20-300 mg/tablet; Starch 0-50 mg/tablet; Magnesium stearate or stearate acid 0-15 mg/tablet; Silicon dioxide, fumed 0-400 mg/tablet; silicon dioxide, colloidal 0-1 mg/tablet, and lactose 0-100 mg/tablet. The ingredients are blended and compressed to form tablets.

### **Formulation 3**

#### **Suspensions**

[0054] Suspensions are prepared using the following ingredients: SERM 0.5-100 mg/5ml; Isoflavone 0.1-1000 mg/ 5ml; Sodium carboxymethyl cellulose 50-700 mg/5ml; Sodium benzoate 0-10 mg/5ml; Purified water 5 ml; and flavour and colour agents as needed.

### **Formulation 4**

#### **Parenteral solutions**

[0055] A parenteral composition is prepared by stirring 1.5% by weight of active ingredients (SERM and isoflavone wt/wt ratio of from 10:1 to 1:10) in 10% by volume propylene glycol and water. The solution is made isotonic with sodium chloride and sterilised.

## Claims

1. A composition for the treatment of breast cancer comprising a combination of a selective estrogen receptor modulator selected from at least one of raloxifene, droloxifene, toremifene, 4-iodotamoxifen, and idoxifene, and at least one isoflavone selected from genistein, daidzein, biochanin A, formononetin, and their respective naturally occurring glucosides and glucoside conjugates.
2. A composition according to Claim 1, wherein said selective estrogen receptor modulator is present in an amount sufficient to prevent, minimise, or reverse the development or growth of breast cancer upon administration of said compound to said human.
3. A composition according to Claim 1 or Claim 2, wherein from about 0.5 mg to about 500 mg of said selective estrogen receptor modulator is present in said composition.
4. A composition according to Claim 3, wherein from about 5 mg to about 100 mg of said selective estrogen receptor modulator is present in said composition.
5. A composition according to any one of Claims 1 to 4, wherein said isoflavone is present in an amount sufficient to augment prevention or minimisation of development or growth of breast cancer provided by said selective estrogen receptor modulator upon administration of said composition to a human.
6. A composition according to any one of Claims 1 to 5, wherein from about 1 mg to about 1000 mg of said isoflavone is present in said composition.
7. A composition according to Claim 6, wherein from about 10 mg to about 200 mg of said isoflavone is present in said composition.
8. A composition according to any one of Claims 1 to 7, wherein said composition contains from about 1% to about 99% selective estrogen receptor modulator, by weight of biologically active ingredients, and said composition contains from about 1% to about 99% isoflavone by weight of biologically active ingredients.
9. A composition according to any one of Claims 1 to 8, further comprising an excipient.
10. A composition according to Claim 9, wherein said selective estrogen receptor modulator, said isoflavone, and said excipient are combined into a pharmaceutical preparation.
11. The use of a selective estrogen receptor modulator selected from at least one of raloxifene, droloxifene, toremifene, 4'-iodotamoxifen, and idoxifene, and an isoflavone selected from at least one of genistein, daidzein, biochanin A, formononetin, and their naturally occurring glucosides and glucoside conjugates for the manufacture of a medicament to prevent, minimise, or reverse the development or growth of breast cancer.
12. A use according to Claim 11, wherein said medicament is so formulated that co-administration of said selective estrogen receptor modulator and said isoflavone is concurrent.
13. A use according to Claim 11, wherein said medicament is so formulated that co-administration of said selective estrogen receptor modulator and said isoflavone is sequential.
14. A use according to any one of Claims 11 to 13, wherein from about 0.5 mg to about 500 mg of said selective estrogen receptor modulator is administered to said human per day.
15. A use according to Claim 14, wherein from about 5 mg to about 100 mg of said selective estrogen receptor modulator is administered to said human per day.
16. A use according to any one of Claims 11 to 15, wherein from about 1 mg to about 1000 mg of said isoflavone is administered to said human per day.
17. A use according to Claim 16, wherein from about 10 mg to about 200 mg of said isoflavone is administered to said human per day.

18. A use according to any one of Claims 11 to 17, wherein said selective estrogen receptor modulator is administered to said human in an amount sufficient to prevent, minimise, or reverse the development or growth of breast cancer.
- 5 19. A use according to any one of Claims 11 to 18, wherein said isoflavone is administered to said human in an amount sufficient to augment prevention, minimisation, or reversal of the development or growth of breast cancer provided by said selective estrogen receptor modulator.
- 10 20. A use according to any one of Claims 11 to 19, wherein said isoflavone is administered to said human in an amount sufficient to prevent or minimise selective estrogen receptor modulator induced uterotrophic effects.
21. A use according to Claim 20, wherein said isoflavone is administered to said human to prevent or minimise the development of endometrial cancer.
- 15 22. A use according to any one of Claims 11 to 21, wherein said selective estrogen receptor modulator and said isoflavone are administered in a pharmaceutical preparation.
23. A use according to Claim 22, wherein said pharmaceutical preparation is a tablet, capsule, powder, suspension, or solution.

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# EUROPEAN SEARCH REPORT

Application Number  
EP 00 30 4697

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	F.SHEN, G.WEBER: "Synergistic down-regulation of signal transduction by tamoxifen and genistein in breast cancer cells" PROCEEDINGS 90TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH APRIL 10-14 1999, vol. 40, 1999, page 496 XP002153096 Philadelphia * page 496 *	1,2,5,8, 11,18-21	A61K31/445 A61K31/35 A61P35/00
X	M.A.ANZANO E.A.: "Chemoprevention of mammary carcinogenesis in the rat: combined use of 9-cis retinoic acid, tamoxifen and other agents" PROCEEDINGS 91TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH APRIL 1-5, 2000, vol. 41, 2000, page 56 XP002153097 San Francisco * page 56 *	1,2,5,8, 11,18-21	
X	F.SHEN, X.XUE, G.WEBER: "Tamoxifen and genistein synergistically down-regulate signal transduction and proliferation in estrogen receptor-negative human breast carcinoma MDA-MB-435 cells" ANTICANCER RESEARCH, vol. 19, no. 3A, 1999, pages 1657-1662, XP000960784 * page 1657 - page 1660 *	1,2,5,8, 11,18-21	A61K
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
Place of search THE HAGUE		Date of completion of the search 16 November 2000	Examiner Peeters, J
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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# EUROPEAN SEARCH REPORT

Application Number  
EP 00 30 4697

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	K.K.CARROLL E.A.: "Anticancer properties of flavonoids, with emphasis on citrus flavonoids" ANTIOXID. HEALTH DIS., vol. 7, 1998, pages 437-446, XP000960909 * page 437 * * page 442 * * page 443 *	1,2,5,8, 11,18-21	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>16 November 2000</b>	Examiner <b>Peeters, J</b>
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application I : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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